(13) + (18) (AA)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 October 2000 (05.10.2000)

PCT

(10) International Publication Number
WO 00/57865 A3

(51) International Patent Classification[7]: A61K 31/665, A61P 43/00, 35/00, 35/02, 3/10, C07F 9/6574

(21) International Application Number: PCT/IL00/00185

(22) International Filing Date: 24 March 2000 (24.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
129178 25 March 1999 (25.03.1999) IL

(71) Applicant *(for all designated States except US)*: YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; at the Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: SHINITZKY, Meir [IL/IL]; 20 Derech Haganim Street, 46910 Kfar Shmaryahu (IL).

(74) Agent: REINHOLD COHN AND PARTNERS; P.O. Box 4060, 61040 Tel Aviv (IL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

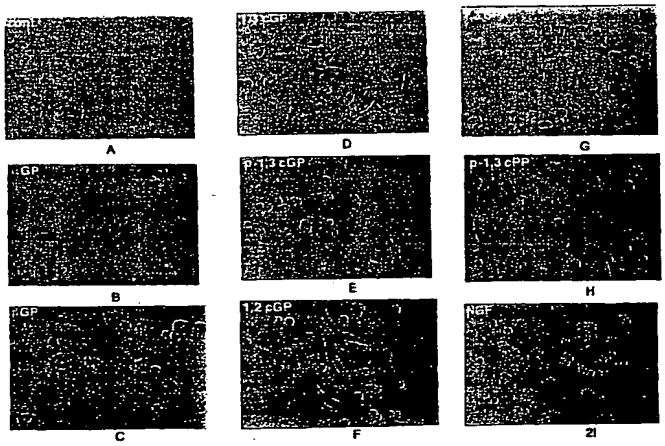
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(88) Date of publication of the international search report:
28 June 2001

[Continued on next page]

(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING CYCLIC GLYCEROPHOSPHATES AND ANALOGS THEREOF FOR PROMOTING NEURAL CELL DIFFERENTIATION



(57) Abstract: Cyclic glycerophosphates and analogs thereof (CGs) are shown to exert neural promoting activities in target cells. Such activities include promotion of neuronal outgrowth, promotion of nerve growth, provision of dopaminotrophic supporting environment in a diseased portion of the brain, prevention of nerve degeneration and nerve rescue. These activities of the CGs render them useful for treatment of various disorders including but not limited to mental disorders such as, for example, schizophrenia, dementia or disorders resulting in learning disablities. In addition, these CGs may be used for the treatment of neurodegenerative conditions such as Altzheimer's diesease, Parkinson's disease, conditions resulting from exposure to harmful environmental factors or resulting from a mechanical injury. The CGs may also be used to treat an individual suffering from a primary neurodengenerative condition in order to prevent or reduce the appearance of secondary degeneration in additional nerves ("*nerve rescue*").

WO 00/57865 A3

INTERNATIONAL SEARCH REPORT

Interr. ial Application No
PCT/IL 00/00185

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/665 A61P43/00 A61P35/00 A61P35/02 A61P3/10
C07F9/6574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, MEDLINE, BIOSIS, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	KINOR N ET AL: "Cyclic glycerophosphates for the treatment of Parkinson's disease" NEUROSCI. LETT., vol. 54, no. supp, November 1999 (1999-11), page S24 XP002155523 abstract ---	1-35
P,X	WO 00 09139 A (ALLELIX BIOPHARMA ;BEGLEITER LEATH E (CA); WICKENS PHILIP L (CA);) 24 February 2000 (2000-02-24) abstract page 11, line 11 -page 12, line 10 page 13, line 26 -page 14, line 22; claims; example 1 --- -/--	1-35

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 December 2000	28/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Orviz Diaz, P

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Preparation of 1-O-acylglycerol-2,3-phosphates and DNA polymerase.alpha. inhibitors containing them" retrieved from STN Database accession no. 124:76506 XP002148571 abstract & JP 07 258278 A (SAGAMI CHEM RES, JAPAN) 9 October 1995 (1995-10-09) ---	1-13
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Method for preparation of 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:144502 XP002148572 abstract & JP 06 228169 A (SAGAMI CHEM RES, JAPAN) 16 August 1994 (1994-08-16) ---	1-13
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Promoters of protein phosphokinase C activation containing 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:350234 XP002148573 abstract & JP 07 149772 A (SAGAMI CHEM RES, JAPAN) 13 June 1995 (1995-06-13) ---	1-13
X	US 5 565 439 A (PIAZZA GARY A ET AL) 15 October 1996 (1996-10-15) abstract column 1, line 60 -column 2, line 39; claims; example II ---	1-13
X	D.C. AYRES ET AL.: "The Organic Chemistry of Phosphorus. Part V." J. CHEM. SOC., 1957, pages 1109-1114, XP000946300 see compounds (VI) and (VII) pages 1111 and 1114 ---	1-13

-/--

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter ...al Application No
PCT/IL 00/00185

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0009139 A	24-02-2000	US 6150345 A AU 5473599 A	21-11-2000 06-03-2000
WO 0057864 A	05-10-2000	NONE	
JP 9025235 A	28-01-1997	NONE	
JP 7258278 A	09-10-1995	NONE	
JP 6228169 A	16-08-1994	NONE	
JP 7149772 A	13-06-1995	NONE	
US 5565439 A	15-10-1996	NONE	

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 October 2000 (05.10.2000)

PCT

(10) International Publication Number
WO 00/57865 A3

(51) International Patent Classification[7]: A61K 31/665, A61P 43/00, 35/00, 35/02, 3/10, C07F 9/6574

(21) International Application Number: PCT/IL00/00185

(22) International Filing Date: 24 March 2000 (24.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
129178 25 March 1999 (25.03.1999) IL

(71) Applicant *(for all designated States except US)*: YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; at the Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: SHINITZKY, Meir [IL/IL]; 20 Derech Haganim Street, 46910 Kfar Shmaryahu (IL).

(74) Agent: REINHOLD COHN AND PARTNERS; P.O. Box 4060, 61040 Tel Aviv (IL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
28 June 2001

(48) Date of publication of this corrected version:
15 November 2001

(15) Information about Correction:
see PCT Gazette No. 46/2001 of 15 November 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/57865 A3

(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING CYCLIC GLYCEROPHOSPHATES AND ANALOGS THEREOF FOR PROMOTING NEURAL CELL DIFFERENTIATION

(57) Abstract: Cyclic glycerophosphates and analogs thereof (CGs) are shown to exert neural promoting activities in target cells. Such activities include promotion of neuronal outgrowth, promotion of nerve growth, provision of dopaminotrophic supporting envrionment in a diseased portion of the brain, prevention of nerve degeneration and nerve rescue. These activities of the CGs render them useful for treatment of various disorders including but not limited to mental disorders such as, for example, schizophrenia, dementia or disorders resulting in learning disablities. In addition, these CGs may be used for the treatment of neurodegenerative conditions such as Altzheimer's diesease, Parkinson's disease, conditions resulting from exposure to harmful environmental factors or resulting from a mechanical injury. The CGs may also be used to treat an individual suffering from a primary neurodengenerative condition in order to prevent or reduce the appearance of secondary degeneration in additional nerves ("*nerve rescue*").

Knusel, *et al., Proc. Natl., Acad. Sci.* USA, **88**:961-965 (1991).

Leloir, L.F., *Biochem. Biophys., J.*, **33**:186 (1951).

Linn, L.F.H., *et al., Science*, **260**:1130-1134 (1993).

Markham, R. and Smith, J.D., *Biochem. J.*, **52**:552- (1952).

Shinitzky, M., Friedman, P. and Haimovitz, R., Formation of 1,3-cyclic glycerophosphate by the action of phospholipase C on phosphatidylglycerol, *J. Biol. Chem.*, **268**:14109-14115 (1993).

Su, B., Kappler, F., Szwergold, B.S. and Brown, T.R., *Cancer Res.*, **53**:1751-1754, (1993).

Tomac, A., *et al., Nature*, **373**:335-339 (1995).

Ukita, T., Bates, N.A. and Carter, H.E., *J. Biol. Chem.*, **216**:867-874, (1955).

BACKGROUND OF THE INVENTION

L-α-glycerophosphate (αGP), a key constituent in phospholipid metabolism (Kennedy and Weiss, 1956), is abundant in most biological tissues (Dawson, 1958). β-Glycerophosphate (βGP) is a product of enzymatic (Ukita *et al.*, 1955) and alkaline (Clarke and Dawson, 1976) hydrolysis of phospholipids and is formed through the cyclic phosphodiester intermediate 1,2-cyclic glycerophosphate (1,2 cGP) (Ukita *et al.*, 1955; Clarke and Dawson, 1976). 1,2 cGP has been detected in algae species (Boyd *et al.*, 1987) as well as in human cancer tissues (Su et al., 1993). Similarly, αGP can in principle adopt the cyclic form 1,3-cyclic glycerophosphate (1,3 cGP). This compound has been shown to be formed as an intermediate in the phospholipase C hydrolysis of phosphatidyl glycerol (PG) (Shinitzky *et al.*, 1993) and upon further hydrolysis is converted to αGP.

A six-membered cyclic phosphate of foremost biological importance is cyclic AMP. The ring of cyclic AMP is actually a derivative of 1,3 cGP backbone. Other cyclic phosphates which were detected in biological systems

List of compounds and their abbreviations

The following compounds which formulas are presented in **Appendix A** just before the claims, will be represented herein in the specification by their abbreviations as follows:

1. 1,3 cyclic glycerophosphate - **1,3 cGP**
2. 1,2 cyclic glycerophosphate - **1,2 cGP**
3. 3-acyl 1,2 cyclic glycerophosphate (cyclic lysophosphatidic acid) - **c-lysoPA**
4. Phenyl 1,3 cGP – **P-1,3 cGP**
5. Phenyl 1,2 cGP – **P-1,2 cGP**
6. 1,3 cyclic propanediol phosphate – **1,3 cPP**
7. 1,2 cyclic propanediol phosphate – **1,2 cPP**
8. Phenyl 1,3 cPP – **P-1,3 cPP**
9. Phenyl 1,2, cyclic propanediol phosphate – **P-1,2, cPP**
10. Cyclic dihydroxyacetone phosphate – **cDHAP**
11. Phenyl cyclic dihydroxyacetone phosphate - **P-cDHAP**

GLOSSARY

The following is an explanation of some terms used above and in the following description and claims:

CG – the cyclic glycerophosphates and analogs thereof used in the present invention.

Promoting neural cell differentiation – this term relates to the capability of the CGs used in the invention to cause cells to mature into neural cells after contact therewith. Such activity may be assessed by one of many *in vitro* and *in vivo* assays such as those described in the examples below. An example for an *in vitro* assay would be to grow cells capable of differentiating into nerve cells (e.g. PC12 cells) in the presence of a tested compound and to determine nerve outgrowth in

compositions or methods are intended for treatment of an ongoing non-desired condition, the term *"effective amount"* should then be understood as meaning an amount of the active compound which is effective in ameliorating or preventing the enhancement of the treated condition and related symptoms.

Neural promoting activity – this term encompasses a variety of neural related activities which may be promoted in target cells upon their contact with the CGs used in the invention. Such activities include but are not limited to promotion of nerve growth, provision of dopaminotrophic supporting environment in a diseased brain, prevention of nerve degeneration, and nerve rescue.

Prevention or treatment – the term prevention of disorders and diseases is to be understood in accordance with the invention as a reduction in the probability of the appearance of such disorders and diseases in an individual having a high predisposition of developing such disorders and diseases, reducing the extent of the symptoms associated with such disorders and diseases when they occur or completely preventing their appearance.

Treatment of such disorders or diseases in accordance with the invention means ameliorating the symptoms associated with the disorders or diseases, reducing the extent of such symptoms or completely eliminating them.

SUMMARY OF THE INVENTION

In accordance with the invention it has surprisingly been found that 1,2 cGP, 1,3 cGP and some of their analogs are capable of promoting neuronal outgrowth of PC12 adrenal tumorigenic cells in culture after a short incubation period.

The present invention thus provides, by a first of its aspects, a pharmaceutical composition for promoting neural cell differentiation in target cells comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general formula I:

dihydroxyacetone phosphate (cDHAP) or phenyl cyclic dihydroxyacetone phosphate (P-cDHAP).

In a further embodiment, Y is $-(CH_2)_m-$, m is 0, X is $-CH_2OH$ and R is H or phenyl. According to this embodiment, the composition comprises 1,2 cyclic glycerophosphate (1,2 cGP) or phenyl 1,2 cyclic glycerophosphate (P-1,2 cGP).

In still a further embodiment, Y is $-(CH_2)_m-$, m is 0, X is a $C_1 - C_{24}$ alkyl, preferably $-CH_3$, and R is a cation or phenyl. According to this embodiment, the composition comprises 1,2 cyclic propanediol phosphate (1,2 cPP) or phenyl 1,2 cyclic propanediol phosphate (P-1,2 cPP).

In yet still a further embodiment, Y is $-(CH_2)_m-$, m is 1, X is a $C_1 - C_{24}$ alkyl, preferably $-CH_3$, and R is a cation or phenyl. According to this embodiment, the composition comprises 1,3 cyclic propanediol phosphate (1,3 cPP) or phenyl 1,3 cyclic propanediol phosphate (P-1,3 cPP).

In yet another embodiment, Y is $-(CH_2)_m-$, m is 0, X is $-CH_2$ (C_1-C_{24})acyl, preferably oleyl, and R is a cation. According to this embodiment, the composition comprises 3-acyl- 1,2 cyclic glycerophosphate (cyclic lisophosphatidic acid – c-lyso PA).

The CGs used in the invention may exert one of many neural promoting activities including but not limited to promotion of neuronal outgrowth, promotion of nerve growth, provision of dopaminotrophic supporting environment in a diseased portion of the brain, prevention of nerve degeneration and nerve rescue. All these activities fall within the scope of neural promoting activity.

Thus, the present invention also provides a pharmaceutical composition for promoting neural activity comprising a pharmaceutical acceptable carrier and, as an active ingredient, a compound of the general formula I above.

The ability of the pharmaceutical compositions of the invention to promote neural cell differentiation and neuronal activity in one or more of the above ways renders them extremely useful for treatment of various disorders. Thus, the invention also provides a pharmaceutical composition comprising a

some known factors which affect neural cells such as NGF, the period of time required for the CGs used in the invention to be in contact with the target cells in order to exert their effect is very short (several minutes).

In accordance with an additional aspect of the invention, a method is provided for promoting neural activity in an individual comprising administering to the individual in need an effective amount of a compound of the general Formula I above.

A method for the prevention or treatment of disorders and diseases which can be prevented or treated by promoting neural cell differentiation and/or neural activity is also provided comprising administering to a person in need a therapeutically effective amount of a compound of Formula I above.

The method of the invention may be used for the treatment of a variety of disorders and diseases in which the abovementioned effects are beneficial, i.e., in which the effect of the CGs ameliorates or reduces the undesired symptoms of the treated condition or disease. These conditions and disorders may be for example, but not limited to, mental disorders such as schizophrenia or dementia, disorders leading to learning disabilities, neurodegenerative disorders such as Alzheimer or Parkinson disease and for prevention or treatment of nerve rescue following nerve injury.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows photographs of PC12 cells following their incubation for 48 hours with growth medium containing linear α glycerophosphate as control (Fig. 1A), with nerve growth factor (NGF) at a concentration of 50 ng per/ml (Fig. 1B) and with 1,3 cyclic glycerophosphate (1,3 GP) at a concentration of 1 μM (Fig. 1C). Neuronal outgrowth is clearly seen in Figs. 1B and 1C.

Fig. 2 shows photographs of PC12 cells grown in culture medium (control) (Fig. 2A), pulsed for three hours with linear α and β glycerophosphates (Fig. 2B and 2C, respectively) with the cyclic glycerophosphates and analogs 1,3 cGP, phenyl-1.3 cGP, 1,2 cGP, 1,3 cPP, and phenyl-1,3 cPP (Fig. 2D – Fig. 2H

preparing the cyclic phosphates of the invention are described specifically below (see Examples).

Analogs of these cyclic glycerophosphates of the invention are also within the scope of the invention being typically deoxy analogs as well as phenyl esters of the 1,3 cyclic phosphates. These analogs may also be prepared by enzymatic methods or synthetically by any of the methods known in the art.

In addition to the active ingredient, the pharmaceutical compositions may also contain a carrier selected from any one of the carriers known in the art. The nature of the carrier will depend on the intended form of administration and indication for which the composition is used. The compositions may also comprise a number of additional ingredients such as diluents, lubricants, binders, preservatives, etc.

The compositions of the invention may be administered by any suitable way. A preferred mode of their administration is either i.v., topically or per os although at times it may be advantageous to use other administration modes as well.

Typically, the pharmaceutical compositions of the invention will comprise about 1 mg to about 10 mg of the active material per kg body weight of the treated individual.

While the compositions of the invention will typically contain a single CG, it is possible at times to include in the composition or to co-administer two or more CGs which may then act together in a synergistic or additive manner to prevent or treat the neurogenerative disorder.

The CGs used in the invention may be used in any of their isomer forms, (see for example, the four stereoisomers which constitute the synthetic 1,3 cGP depicted in Appendix A). For various purposes, one of the isomers may be preferred over the remaining ones.

According to the invention, the CGs may be administered either in a single dose or may be given repetitively over a period of time.

The compositions of the invention may also be administered to the treated individual in combination with an additional treatment, e.g. wherein the treated

Example 1: Synthesis of 1,3 cyclic glycerophosphate (1,3 cGP)

The procedure of Buchnea (Buchnea, 1973) was followed essentially as described. Briefly, 2-benzyloxy-1,3-propanediol (Aldrich) was reacted with an equimolar amount of phosphorus oxychloride (Aldrich) in methylene chloride. The resulting 2-benzyl-1,3 cGP was treated with hydrogen under the catalysis of Pd black in methanol to remove the benzyl residue. The 1,3 cGP, isolated as the Ba salt, was pure on paper chromatography (n-propanol: ammonia: water 6:3:1, R_f=0.52).

1,3 cGP was also produced by the cleavage of phosphatidyl glycerol (PG) with phospholipase C as described (Shinitzky et al., 1993). The product had a trace of approx. 10-20% α-GP as indicated by paper chromatography.

Example 2: Synthesis of 1,2 cyclic glycerophosphate (1,2 cGP)

This compound was prepared as described (Kugel, L. and Halmann, M., *J. Am. Chem. Soc.*, **89**:4125-4128 (1967). The disodium salt of β-glycerophosphate (Sigma) was first converted to the acid form and then cyclized with dicyclohexylcarbodiimide (Aldrich). The product, isolated as the Ba salt, was pure on paper chromatography.

Example 3: Synthesis of phenyl 1,3 cyclic glycerophosphate (P-1,3 cGP)

The method described in Example 1 for 1,3 cGP was followed by reacting 2-benzyloxy-1,3-propanediol with phenyl phosphorodichloridate (Aldrich). The intermediate benzylated product was pure on thin layer chromatography (ethyl acetate:hexane 3:2 R_f =0.58), with a melting point of 136°C. It was further hydrogenated as in Example 1 to remove selectively the benzyl residue. The obtained P-1,3 cGP, compound **III**, was pure on thin layer chromatography (as above) with R_f =0.15 and melting point of 116°C.

Example 4: Synthesis of 1,3 cyclic propanediol phosphate (1,3 cPP)

1,3 cPP was prepared by reacting 1,3-propanediol (Aldrich) with an equimolar amount of phosphorus oxychloride and then purified as described by

Crystallization was achieved from ethyll acetate-hexane and the product had a melting point of 69°C.

Example 9: Synthesis of cyclic dihydroxyacetone phosphate (cDHAP)

This novel compound was prepared by reaction of $POCl_3$ with dihydroxyacetone.

1.8 g (0.01M dimer or 0.02M monomer) Dihydroxyacetone dimer MW-180 dissolved in 20 ml fresh distilled methylene chloride.

3.07 g = 1.87 ml (0.02M) Phosphoryl chloride (MW-153.5, d-1.645) in 4 ml $MeCl_2$ was slowly added to the solution at RT. The solution was refluxed for 15 h (the solution was black). Methylene chloride was evaporated and 100 ml 90% acetone/water was added to the solution. The reaction mixture was refluxed for 18 h. The black solution was treated with active carbon at RT and filtered. From the resulting slightly yellow solution was evaporated acetone and water and the very nice crystalline residue was dissolved in 10 ml acetone. 0.01 M BaJ_2 in 80 ml acetone was added to the solution and nice crystals of cyclic-dihydroxyacetone-phosphate barium salt started to precipitate. The precipitate was washed 3 times with small quantities of acetone and dried. The product was cleaned by dissolving it in small amounts of water and precipitating with acetone. The resulting produce is white crystalline powder and shows in paper chromatography (solvents mixture: n-Propanol:NH_4H_2O 6:3:1) R_f – 0.50.

Example 10: Synthesis of phenyl cyclic dihydroxyacetone phosphate (P-cDHAP)

This novel compound was prepared by reaction of phenyl-PO_2Cl_2 with dihydroxyacetone in dry pyridine. Upon removal of the solvent by vacuum, the residue was extracted twice with ethyl acetate. After evaporation of the ethyl acetate, an oily residue was obtained..

rotation in rats with unilateral dopaminergic ablation, with rotation occurring in the direction contralateral to the side of the lesion.

Administration of cyclic glycerophosphates and analogs thereof into the brain

Cyclic phosphates are administered into the brain using ALZET osmotic pumps (ALZET Corporation, Palo Alto, CA). A canulla (30 gauge) is implanted 0.5 mm medial to the SN of rats, using a stereotaxic device after assessment of nigrostriatal lesions (rotation behavior). Cyclic phosphates are microperfused at a rate of 1 μl/h for 3 or 14 days.

Brain dissection and extraction

Rats are decapitated and their brains rapidly removed. The brains are then placed in a rat brain mold on ice and 0.5 mm serial sections are cut and placed on chilled microscope slides. Tissue punches are rapidly taken using a stainless steel cannula with an inner diameter of 1.1 mm, according to the following coordinates: A1.5-2.0 mm for the striatum; P5.5-5.0 mm for the SN, and include most of the desired regions. The tissue samples are immediately frozen in liquid nitrogen and stored at -70°C until extraction. Extraction is achieved by thawing the punches and subjecting them to probe sonication (80 watts for 5 sec. with a Sonifier B-12; Branson, Danbury, CN) in 0.5 ml of a perchlorate solution (0.1M) containing EDTA/ethanol (0.021%) on ice. A sample (100 μl) is removed for protein analysis and the remainder is centrifuged (2000 x g, 10 mins. 4°C). The resulting supernatants (the tissue extracts) are filtered (0.45 μm Acrodisk, Gelman; Ann. Arbor. MI) and stored at -70°C until subjected to ELISA analysis to determine ILS or GDNF or HPLC analysis to determine the 5-HT and 5-HIAA content.

5′-TACATCCACACCTTTTAGCG -3′{3′} corresponding to bases 81-101 and 460-480 respectively) (Biosource, CA, USA), and 2.5 U Taq DNA polymerase (Boehringer Mannheim). Reactions are overlaid with mineral oil, and initially denatured at 94°C for 2 min. PCR is performed using a MJ Research thermal cycler programmed for 40 cycles consisting of denaturation at 94°C for 1 min. followed by primer annealing at 55°C for 1 min. and primer extension at 72°C for 1 min. At the end of the 40 cycles, the reaction mixture is kept at 72°C for 10 min. The PCR product is electrophoretically analyzed on a 2% agarose gel containing ethidium bromide).

Immunohistochemical assessment of the cell survival in the brain

At the end of the experiment the animals are anesthetized with ketamine and xylazine i.p. and then perfused via cardiac puncture with PBS followed by 4% paraformaldehyde. The brains are then removed and post-fixed in 4% paraformaldehyde for 24 hrs and then transferred into 20% sucrose for 48 hours. Tissue sections of 35 μm are obtained using a cryostat and placed in 24 wells plate in PBS. The sections are incubated overnight in 4°C with a primary rabbit polyclonal antibody to Tyrosine hydroxylase (TH) (Chemicon, CA, USA) or a primary mouse monoclonal antibody to glial fibrillary acidic protein (GFAP) (Chemicon, CA, USA). The sections are then washed with PBS, incubated (1 hr) with a HRP conjugated secondary antibody (sheep anti-rabbit or anti-mouse) (Chemicon, CA, USA) and washed with PBS. Then, the sections are incubated with the chromagen diaminobenzidine (DAB), counter-stained with hematoxylin, and screened by light microscopy. Positive staining for TH indicates the amount of dopaminergic- cells in the striatum and substantia nigra, i.e. dopaminergic-cells survival. Positive staining for the GFAP in the injection tract indicates glioma processes.

RESULTS

Example 12

PC12 cells were grown in culture as explained above, the cells were divided into three groups and different factors were added to the growth medium of each group for 48 hours as follows:

Group A - α glycerophosphate.

Group B - nerve growth factor (NGF) at a concentration of 50 ng/ml.

Group C – 1,3 cGP at a concentration of 1 μM.

The rate of neuronal growth in each of the above cultures was monitored and documented by microscopic photographs. As seen in Fig. 1, while growth of the cells in the presence of α glycerophosphate did not promote neural outgrowth in the cells (Fig. 1A) such neural outgrowth was clearly seen in the cells which were grown in the presence of NGF (Fig. 1B) or 1,3 cGP (Fig. 1C).

Example 13

Cells were grown as described in Example 12 above with the same factors and at distinct stages the level of intercellular signaling proteins were evaluated by a Western Blot technique using antibodies specific for the tested proteins.

Example 14

Cells were grown as described above and divided into groups which were each grown with one of the following:

(A)	growth medium	(B)	αGP	(C)	βGP
(D)	1,3 cGP	(E)	phenyl 1,3 cGP	(F)	1,2 cGP
(G)	1,3 cPP	(H)	phenyl-1,3 cPP	(I)	NGF

The above factors were added to the cells for a period of three hours after which they were washed away from the cells. The cells were further grown in a growth medium which did not comprise the above factors. The neural outgrowth of

Example 17

Parkinson's disease is induced in rats as described in the Materials and Methods part above by injection of 6OH-DA into their brains.

The rats are then treated either with α and β linear GPs or with CG by administration of the either topically, per os, or directly into the brain using osmotic pumps as described above.

The effect of the linear GPs and of the CGs is assessed by evaluating the *in situ* production of L-DOPA, dopamine (DA), the dopamine metabolites DOPAC and HVA and the growth factor GDNF by using microdialysis techniques and by the methods described above. Motional and limb tremor parameters are also quantitatively evaluated in the rats treated with each of the above factors.

Example 18

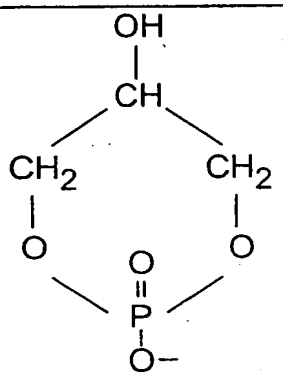
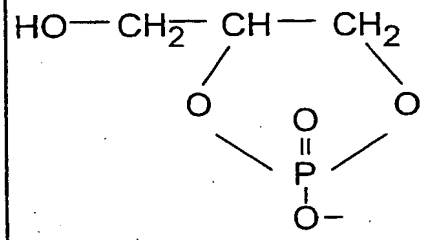
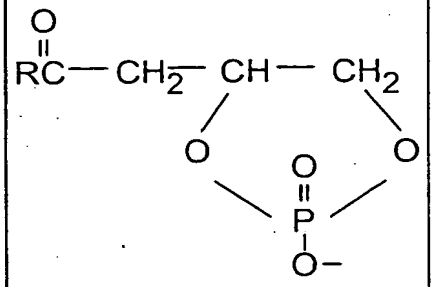
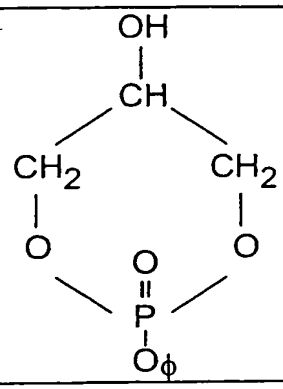
Rats having injured optical nerves are treated with α and β linear glycerophosphates or with a CG as explained above and the effect of the above CG on the visual response and nerve generation of the treated rats is monitored.

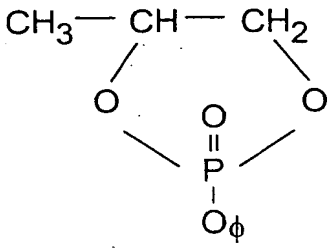
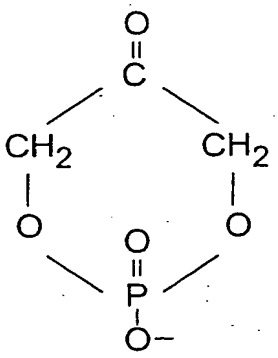
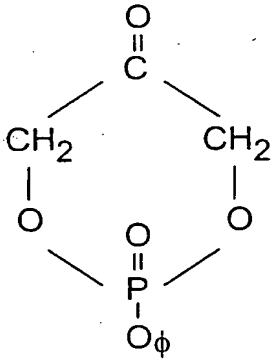
Example 19

To study nerve rescue by 1,3 cPP, PC12 cells were incubated in tissue culture medium for a period of 14 days. Within this period, the cells were either grown in the presence of nerve growth factor (NGF) for different periods of times or were grown in the presence of 1,3, cPP for various periods of time. Neuronal differentiation and spread was examined in the various cells.

As seen in Fig. 4A, wherein the PC12 cells were grown in growth medium with no additives added, no neuronal spreading was observed (control). Growth of the cells in the presence of NGF (50 ng/ml) for the full period of 14 days resulted in full neuronal differentiation as seen in Fig. 4B. As seen in Fig. 4C, when the cells were grown for the first 7 days in the presence of NGF (50 ng/ml) and then cultured without NGF for an additional period of 7 days, complete nerve retraction was observed and the level of differentiation of the cells returned to control level.

Appendix A

	Formula	Abbreviation
I		1,3 cGP
II		1,2 cGP
III		cyclic lysophosphatidic acid, c-lypoPA
IV		P-1,3 cGP

	Formula	Abbreviation
IX		p-1,2 cPP
X		cDHAP
XI		P-cDHAP

5. A pharmaceutical composition according to Claim 4, wherein said disorders and diseases are mental disorders.

6. A pharmaceutical composition according to Claim 5, wherein said mental disorder is schizophrenia or dementia.

7. A pharmaceutical composition according to Claim 5, wherein said mental disorder is a learning disability.

8. A pharmaceutical composition according to Claim 4, for the treatment of neurodegenerative conditions involving damage to the dopaminergic neural cells.

9. A pharmaceutical composition according to Claim 8, wherein said neurodegenerative condition is Alzheimer's disease.

10. A pharmaceutical composition according to Claim 8, wherein said neurodegenerative condition is Parkinson's disease.

11. A pharmaceutical composition according to Claim 4, wherein said disorders and diseases result from exposure to harmful environmental factors or from a mechanical injury.

12. A pharmaceutical composition according to Claim 4, for the treatment of nerve rescue after nerve injury.

13. A pharmaceutical composition according to any one of Claims 1-12, wherein the active ingredient is a compound of Formula I selected from the group consisting of:

i. 1,3 cyclic glycerophosphate - **1,3 cGP;**

ii. 1,2 cyclic glycerophosphate - **1,2 cGP;**

iii. 3-acyl 1,2 cyclic glycerophosphate (cyclic lysophosphatidic acid) - **c-lysoPA;**

iv. Phenyl 1,3 cGP – **P-1,3 cGP;**

v. Phenyl 1,2 cGP – **P-1,2 cGP;**

vi. 1,3 cyclic propanediol phosphate – **1,3 cPP;**

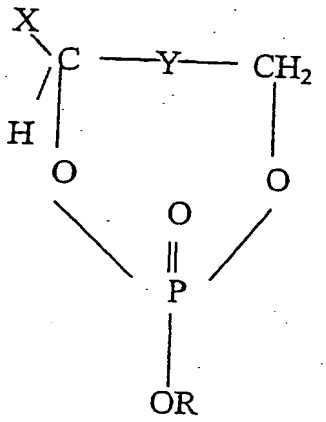
vii. 1,2 cyclic propanediol phosphate – **1,2 cPP;**

viii. Phenyl 1,3 cPP – **P-1,3 cPP;**

ix. Phenyl 1,2, cyclic propanediol phosphate – **P-1,2, cPP;**

24. A method according to Claim 15, for the treatment of nerve rescue after nerve injury.

25. Use of a compound of the general Formula I



wherein

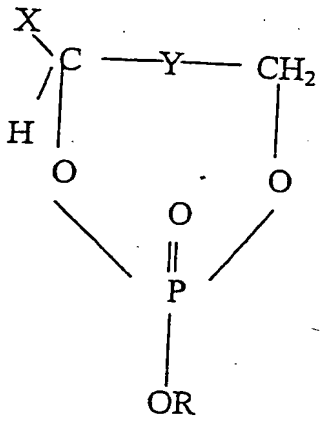
Y is $-(CH_2)_m-$, -CH(OH)- or -C(=O)- , and m is 0 - 3 ;

X is H, alkyl, $-CH_2OH-$, CH_2Oacyl or $-CH_2$acyl; and

R is H, a cation, alkyl or optionally substituted aryl

for the preparation of a pharmaceutical composition for promoting neural cell differentiation.

26. Use of a compound of the general Formula I



wherein

Y is $-(CH_2)_m-$, -CH(OH)- or -C(=O)- , and m is 0 - 3 ;

X is H, alkyl, $-CH_2OH-$, CH_2Oacyl or $-CH_2$acyl; and

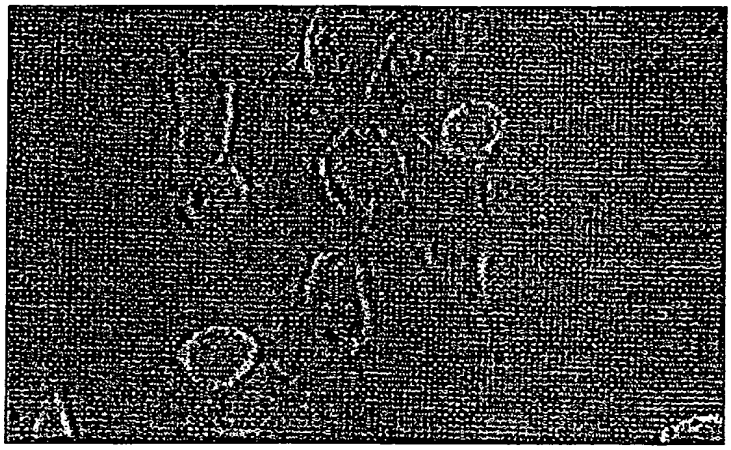


FIG. 1A

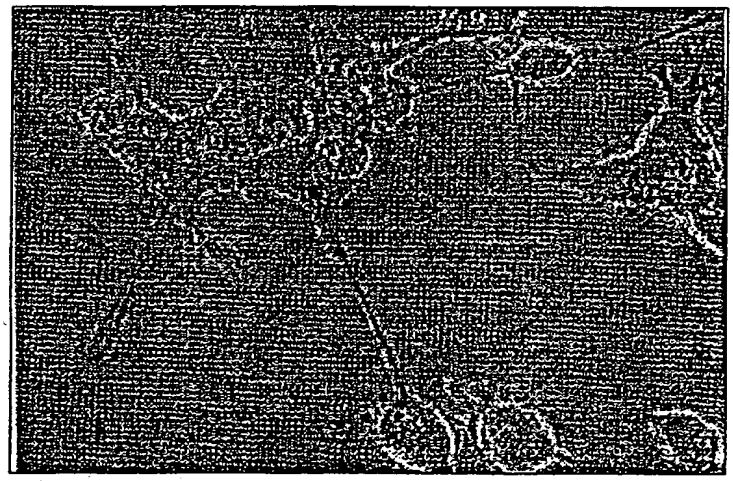


FIG. 1B

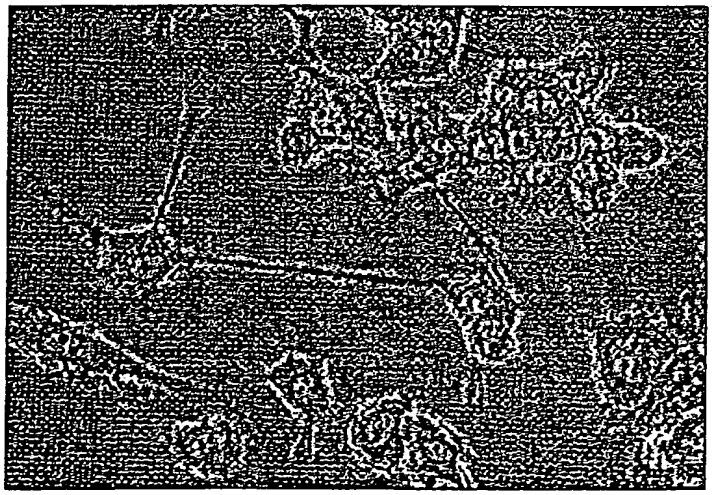


FIG. 1C

FIG. 2D

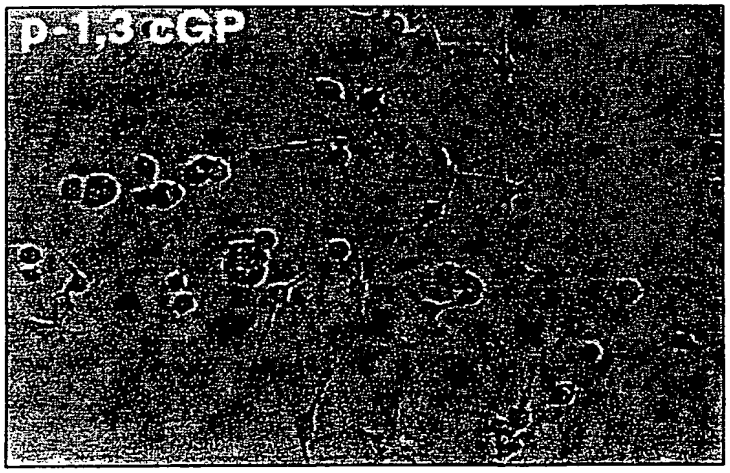


FIG. 2E

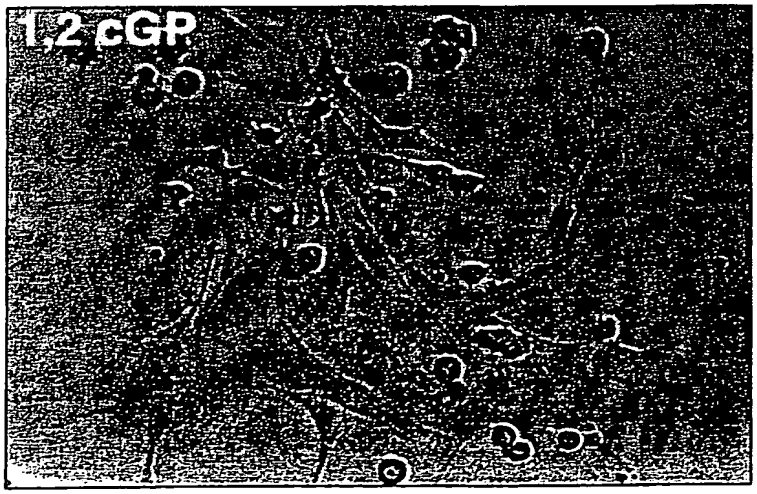


FIG. 2F

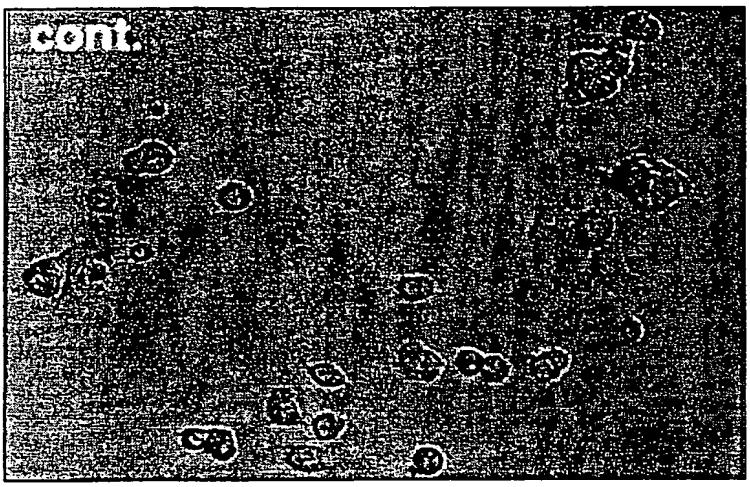


FIG. 3A

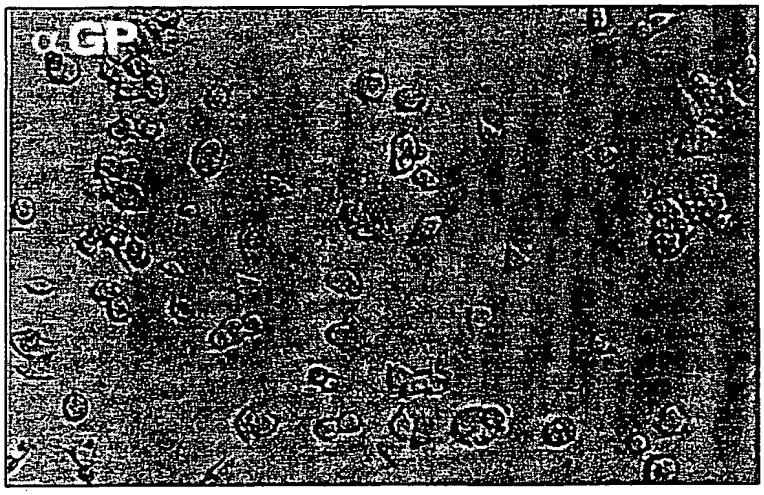


FIG. 3B

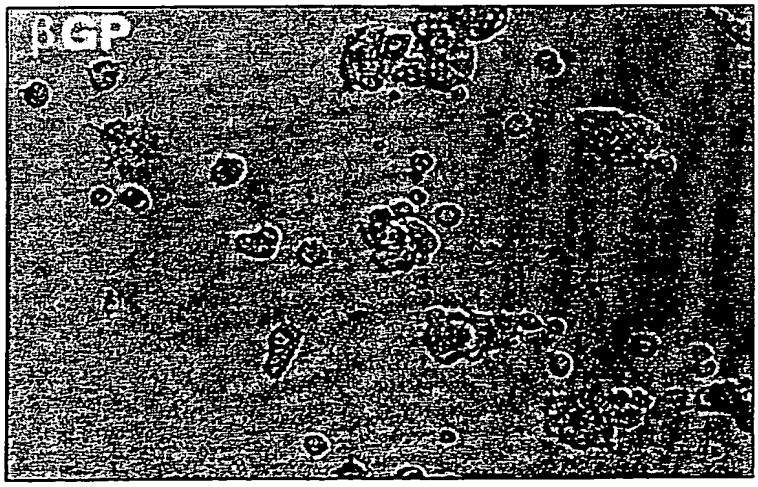


FIG. 3C

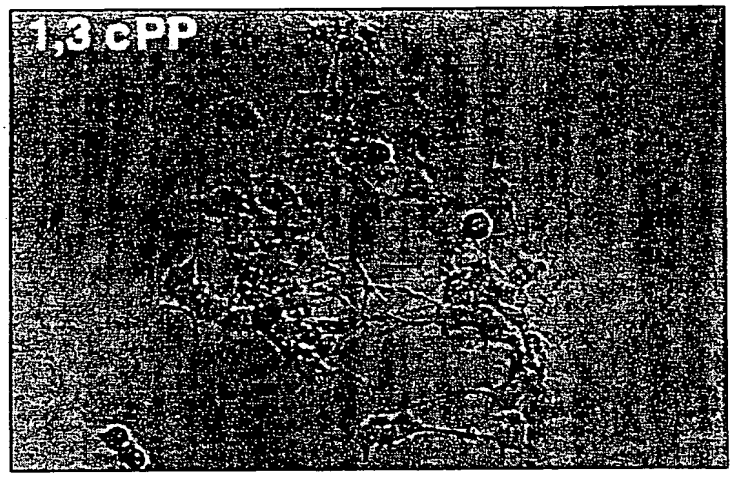


FIG. 3G

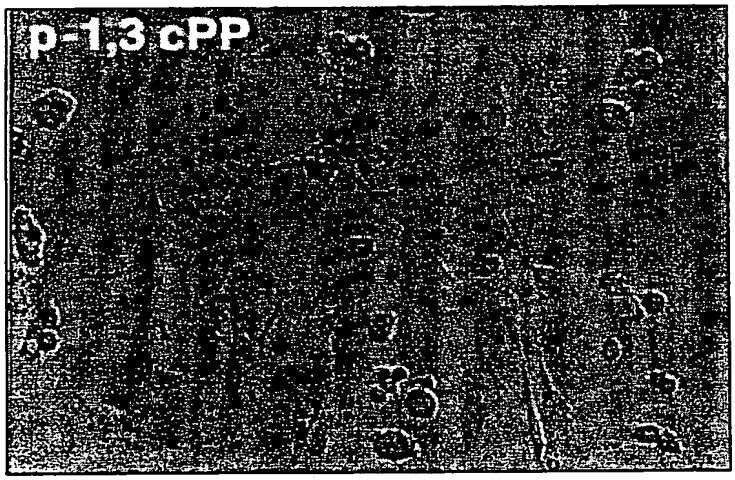


FIG. 3H

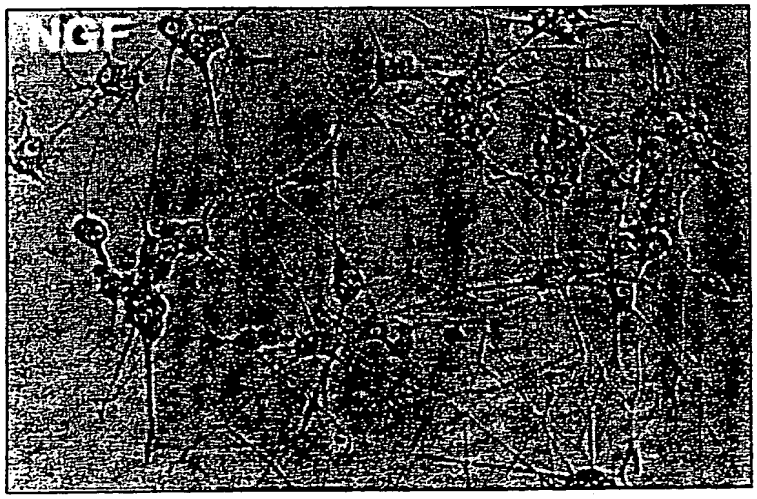


FIG. 3I

INTERNATIONAL SEARCH REPORT

Interr. ial Application No
PCT/IL 00/00185

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/665 A61P43/00 A61P35/00 A61P35/02 A61P3/10
C07F9/6574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, MEDLINE, BIOSIS, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	KINOR N ET AL: "Cyclic glycerophosphates for the treatment of Parkinson's disease" NEUROSCI. LETT., vol. 54, no. supp, November 1999 (1999-11), page S24 XP002155523 abstract --- WO 00 09139 A (ALLELIX BIOPHARMA ;BEGLEITER LEATH E (CA); WICKENS PHILIP L (CA);) 24 February 2000 (2000-02-24) abstract page 11, line 11 -page 12, line 10 page 13, line 26 -page 14, line 22; claims; example 1 --- -/--	1-35 1-35

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 December 2000	28/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Orviz Diaz, P

Form PCT/ISA/210 (second sheet) (July 1992)

SDOCID: <WO___0057865A3_IA>

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Preparation of 1-O-acylglycerol-2,3-phosphates and DNA polymerase.alpha. inhibitors containing them" retrieved from STN Database accession no. 124:76506 XP002148571 abstract & JP 07 258278 A (SAGAMI CHEM RES, JAPAN) 9 October 1995 (1995-10-09) ---	1-13
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Method for preparation of 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:144502 XP002148572 abstract & JP 06 228169 A (SAGAMI CHEM RES, JAPAN) 16 August 1994 (1994-08-16) ---	1-13
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Promoters of protein phosphokinase C activation containing 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:350234 XP002148573 abstract & JP 07 149772 A (SAGAMI CHEM RES, JAPAN) 13 June 1995 (1995-06-13) ---	1-13
X	US 5 565 439 A (PIAZZA GARY A ET AL) 15 October 1996 (1996-10-15) abstract column 1, line 60 -column 2, line 39; claims; example II ---	1-13
X	D.C. AYRES ET AL.: "The Organic Chemistry of Phosphorus. Part V." J. CHEM. SOC., 1957, pages 1109-1114, XP000946300 see compounds (VI) and (VII) pages 1111 and 1114 ---	1-13

-/--

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter ...al Application No
PCT/IL 00/00185

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0009139 A	24-02-2000	US 6150345 A AU 5473599 A	21-11-2000 06-03-2000
WO 0057864 A	05-10-2000	NONE	
JP 9025235 A	28-01-1997	NONE	
JP 7258278 A	09-10-1995	NONE	
JP 6228169 A	16-08-1994	NONE	
JP 7149772 A	13-06-1995	NONE	
US 5565439 A	15-10-1996	NONE	

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.